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Performance of the Cepheid CT/NG Xpert Rapid PCR Test for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, which can provide results rapidly to guide therapeutic decision-making, offer patient care advantages over laboratory-based tests that require several days to provide results. We compared results from the Cepheid GeneXpert CT/NG (Xpert) assay to results from two currently approved nucleic acid amplification assays in 1,722 female and 1,387 male volunteers. Results for chlamydia in females demonstrated sensitivities for endocervical, vaginal, and urine samples of 97.4%, 98.7%, and 97.6%, respectively, and for urine samples from males, a sensitivity of 97.5%, with all specificity estimates being ≥99.4%. Results for gonorrhea in females demonstrated sensitivities for endocervical, vaginal, and urine samples of 100.0%, 100.0%, and 95.6%, respectively, and for urine samples from males, a sensitivity of 98.0%, with all estimates of specificity being ≥99.8%. These results indicate that this short-turnaround-time test can be used to accurately test patients and to possibly do so at the site of care, thus potentially improving chlamydia and gonorrhea control efforts.

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the agents of the two most prevalent bacterial sexually transmitted infections (STIs) reported to the Centers for Disease Control and Prevention (CDC), accounting for >1.6 million reported infections in the United States in 2010 (1). The CDC estimates that STIs cost the health care system $1.5 billion annually. Since these infections, especially chlamydia, are most often asymptomatic, the CDC recommends yearly screening for chlamydia in all sexually active women aged 16 to 25 years. Further, since coinfections are common, most diagnostic test platforms assay for both organisms. Nucleic acid amplification tests (NAATs) are now recommended by the CDC (2) as the tests of choice; however, current NAATs are classified as being of high or moderate complexity and might take 1 to 2 days for results to become available. New assays and new platforms that provide results at the time of patient visits are urgently needed, since many patients do not return for their results when laboratory-based tests that require several days for their results are performed (3, 4).

The Cepheid GeneXpert CT/NG (Xpert) assay is a rapid (<2 h to results) NAAT assay that can be performed in on-site laboratories. The assay detects the DNA of *C. trachomatis* and *N. gonorrhoeae* from endocervical, vaginal, and urine specimens of females, as well as from urine specimens of males, from both symptomatic and asymptomatic individuals. The Xpert test is performed using a modular cartridge-based platform for testing each specimen by nucleic acid amplification, and it can process from 1 to 96 specimens in <2 h with easy-to-use cartridges that minimize processing steps and contamination. This study compares the clinical performance (as measured by sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV]) of the Xpert assay to the patient infection status (PIS) for chlamydia and gonorrhea in patients from high- and low-prevalence sites.

MATERIALS AND METHODS

We performed a multicenter evaluation of the performance characteristics of the Xpert assay compared to PIS determined using two FDA-cleared NAATs for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Fig. 1) (5). The comparator methods were the Gen-Probe Hologic APTIMA Combo 2 assay (AC2) (Tigris platform; Gen-Probe, San Diego, CA) (6, 7, 8, 9) and the ProbeTec ET *C. trachomatis* and *N. gonorrhoeae* amplified DNA assays (BDPT) (Viper platform; Becton, Dickinson, Sparks, MD) (10, 11). Testing was performed according to the assay package inserts of each manufacturer. All sites obtained institutional review board approval for the trial and conducted the study in accordance with the approved protocol consistent with the principles of good clinical and laboratory practices.

Patient population. The sample size was calculated using the following statistical plan: sensitivity (both genders, all matrix) required ≥95%, and specificity (both genders, all matrix) required ≥98%. The required sample size calculations assumed that subjects would be enrolled from sites with an approximate prevalence range of 5% to 10% for *C. trachomatis* and 3% to 7% for *N. gonorrhoeae*. For each site, male prevalence rates were assumed to be 2% higher than for females.

Specimens were collected from consenting sexually active symptomatic and asymptomatic males and females attending obstetrics and gynecology (OB-GYN), sexually transmitted disease (STD), teen, public health, or family planning clinics. Specimen types included urine from males and females, as well as endocervical swabs and patient-collected vaginal swabs from women (collected from patients in a clinical setting). The urine from each patient was collected as first-catch urine, which was then divided into the three parts for use with the 3 different transport media, according to directions of each manufacturer.

Inclusion criteria for study participation included signed informed consent documents, an age of ≥14 years, sexual activity within the past 6 months, and attending a participating clinic for reasons appropriate for sexual health screening. Exclusion criteria included having been previously en-

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		NAAT 1 Swab	Pos	Pos	Pos	E	E	E	Neg	Neg	Neg
		NAAT 1 Urine	Pos	E	Neg	Pos	E	Neg	Pos	E	Neg
NAAT 2 Swab	NAAT 2 Urine										
Pos	Pos		I	I	I	I	NI	NI	I	NI	NI
Pos	E		I	I	I	I	NI	NI	I	NI	NI
Pos	Neg		I	I	I	I	NI	NI	I	NI	NI
E	Pos		I	I	I	I	NI	NI	I	NI	NI
E	E		NI	NI	NI	NI	EQ	NI	NI	NI	NI
E	Neg		NI	NI	NI	NI	NI	NI	NI	NI	NI
Neg	Pos		I	I	I	I	NI	NI	I	NI	NI
Neg	E		NI	NI	NI	NI	NI	NI	NI	NI	NI
Neg	Neg		NI	NI	NI	NI	NI	NI	NI	NI	NI

FIG 1 Patient infection status algorithm based on comparator assays. NAAT 1 and NAAT 2, AC2 (APTIMA Combo 2) and BDPT (Becton, Dickinson ProbeTec), respectively; I, infected; E, equivocal; NI, not infected; NAAT, nucleic acid amplified test.

rolled in the trial, having received antimicrobial therapy within 21 days preceding enrollment, and (for females) a history of hysterectomy. Minors and pregnant women, who represent the groups that are normally tested for chlamydia and gonorrhea, were not excluded from study participation. All sites adhered to local policies and regulations that govern the enrollment of study participants and particularly for minors or pregnant women.

Patients were classified as symptomatic if they reported any of the following symptoms: dysuria, urethral discharge, coital pain/difficulty/bleeding, testicular or scrotal pain/swelling, abnormal vaginal discharge, or pelvic/uterine/adnexal pain. Subjects without these symptoms were classified as asymptomatic.

Specimen collection. The following specimens were collected for each woman: three endocervical swabs, one patient-collected vaginal swab (only for the Cepheid assay and collected in the clinical setting), and a urine specimen. The endocervical swabs for the two comparator assays were used to determine the PIS.

The order of endocervical specimen collection was randomized, such that swabs for each of the three test assays had equal opportunity to be collected first, second, or third. From each male participant, a urine specimen and two urethral swabs were collected. Two urethral swabs were obtained solely for the determination of the patient infection status (PIS) by the reference methods because the Cepheid Company was seeking FDA clearance only for urine specimens, and not for urethral swabs, in males. The order of specimen collection was randomized, such that each swab type had equal opportunity to be collected first or second. All swab samples were collected and transported according to each manufacturer's package insert directions. A single first-catch urine sample from each participant was aliquoted into each manufacturer's urine collection device or transport kit.

Specimen testing. The AC2 and BDPT assays are FDA-cleared tests and were performed at a central laboratory as reference assays according to each manufacturer's instructions. The AC2 results are qualitative and were reported as positive, presumed negative, or indeterminate for *C. trachomatis* rRNA and as positive, presumed negative, or indeterminate for *N. gonorrhoeae* rRNA. Repeatedly equivocal results were reported as indeterminate. The BDPT *C. trachomatis* and *N. gonorrhoeae* results are also qualitative and were reported as positive, negative, or indeterminate for each organism. Repeatedly inhibitory specimens were reported as indeterminate.

The Xpert test was performed according to the draft package insert instructions. The targets for *N. gonorrhoeae* were two highly conserved noncontiguous chromosomal targets that are unique to *N. gonorrhoeae* and not found in other *Neisseria* species, and the *C. trachomatis* target was a chromosomal target. The GeneXpert system is a closed self-contained fully integrated automated platform that represents a paradigm shift in the automation of molecular analysis, producing results rapidly with a minimal risk of contamination. The GeneXpert system combines onboard sample preparation with real-time PCR amplification and detection functions for fully integrated and automated nucleic acid analysis. The system is designed to purify, concentrate, detect, and identify targeted nucleic acid sequences, thereby delivering answers directly from unprocessed samples. The assay requires three steps: transfer 300 μl of prepared sample into the large hole in the cartridge, dispense elution reagent into the small hole in the cartridge, and insert the cartridge into Xpert platform and start the assay.

Test sites included the study investigational sites, which collected and performed assays, or sites that were collection sites only and sent specimens to another qualified study laboratory to perform the assays. Results included a specimen adequacy control result and an amplification control result. Results were reported as positive or negative for chlamydia, positive or negative for gonorrhea, or indeterminate (reading invalid, error, or no result). If the initial Xpert result was indeterminate, the specimen was retested one time using a new aliquot of specimen, if available, and a new Xpert cartridge. When either of these failed, the test was read as indeterminate and the test was repeated, since both need to be amplified for a valid test result. The adequacy control is quite important, as it ensures that there is sufficient human DNA in the sample; otherwise, the test is reported as indeterminate (i.e., the patient put water or other liquid in the collection cup instead of urine or submitted an "air swab" for the self-collected vaginal swab).

Data analysis and interpretation of assay results. Determination of the patient infection status (PIS) is intended to aid in the estimation of sensitivity and specificity in situations for which a true measure of infection does not exist. Thus, when 3 NAATs are used to test each sample, the estimate of sensitivity for one assay is determined by comparing it to the results obtained by the other assays. Although multiple samples are tested per patient, and any sample type might contribute to defining an individual as infected, patients for this trial were defined as infected for each specimen type if a minimum of one positive result was reported by each of the two comparator NAAT assays for that specimen type; thus, two comparator positives are required for that specimen type, at least one from each comparator assay (Fig. 1).

TABLE 1 Patient characteristics and disease prevalence

Characteristic	No. (%) of participants	Prevalence (% [95% CI])	
		Chlamydia trachomatis	*Neisseria gonorrhoeae*
Sex			
Female	1,722 (55.4)	4.8 (3.8–5.9)	1.3 (0.9–2.0)
Male	1,387 (44.6)	5.8 (4.7–7.2)	3.6 (2.7–4.7)
Symptomatic			
Yes	839 (27.0)	9.9 (8.0–12.1)	6.7 (5.1–8.6)
No	2,270 (73.0)	3.5 (2.8–4.4)	0.7 (0.4–1.2)
Clinic type			
Family planning	510 (16.4)	5.3 (3.5–7.6)	2.0 (0.9–3.6)
Public health	969 (31.2)	7.3 (5.8–9.2)	5.2 (3.9–6.7)
STD[a]	206 (6.6)	12.6 (8.4–17.9)	2.9 (1.1–6.2)
Other	1,424 (45.8)	2.7 (2.0–3.7)	0.5 (0.2–1.0)

[a] STD, sexually transmitted disease.

Rolling patient infection status. In addition to estimating the performance characteristics of the Xpert assay using the PIS, we also compared the performance of each assay relative to that of each of the other two assays used in the study that apply the PIS method (5).

RESULTS

Patient characteristics, clinic types, and disease prevalence for the 1,722 female and 1,387 male subjects tested by the Xpert assay and both comparator tests are shown in Table 1. A total of 82/1,722 (4.8%) female subjects were infected with *C. trachomatis* and a total of 23/1,722 (1.3%) women were infected with *N. gonorrhoeae* (Table 1). Eighty-one of 1,387 (5.8%) men were infected with *C. trachomatis* and a total of 50 (3.6%) men were infected with *N. gonorrhoeae* (Table 1). There were 47 subjects were infected with both chlamydia and gonorrhea. Overall, symptoms were reported in 27% of participants.

Xpert assay results were generated for 97.1% of eligible samples on the first attempt (i.e., the inhibition or inadequate sample rates combined were <3%). One hundred eighty-five of the 190 indeterminate cases (159 error results, 17 invalid results, and 14 no result) were retested, and 164 yielded valid results upon repeat assay. There were only 17 invalid results (i.e., *C. trachomatis* and *N. gonorrhoeae* were negative and sample adequacy control [SAC] and sample process control [SPC] failed due to inhibition, aberrant sample preparation, or inadequate sample), which is about 0.25% from a total of ~6,550 specimens. The overall rate of assay success was 99.6%.

Assay performance compared to PIS. (i) Chlamydial infections. Relative to the PIS, the Xpert CT/NG assay demonstrated a sensitivity and specificity for *C. trachomatis* for patient-collected vaginal swabs of 98.7% and 99.4%, respectively (Table 2). Sensitivity and specificity estimates for *C. trachomatis* for endocervical swabs were 97.4% and 99.6%, respectively. Finally, for urine specimens from females, the sensitivity and specificity for *C. trachomatis* were 97.6% and 99.8%, respectively. Using urine specimens from males, compared to the PIS, the Xpert assay demonstrated a sensitivity and specificity for *C. trachomatis* of 97.5% and 99.9%, respectively.

(ii) Gonococcal infections. For *N. gonorrhoeae*, the sensitivity and specificity using vaginal swabs were 100% and 99.9%, respectively (Table 3). The sensitivity and specificity obtained with endocervical samples were both 100%, and for urine samples from

TABLE 2 Performance of Xpert CT/NG versus patient infection status by symptomatic status for *Chlamydia trachomatis*

Sample type[a]	Status[b]	Total (*n*)	Sens (% [no. positive/no. total])[c]	95% CI	Spec (% [no. positive/no. total])[d]	95% CI	Prev[e] (%)	PPV[f] (%)	NPV[g] (%)
VS	Symp	581	100 (30/30)	90.5 to 100	99.5 (548/551)	98.4 to 99.9	5.2	90.9	100
	Asymp	1,132	98.0 (48/49)	89.1 to 99.9	99.4 (1,076/1,083)	98.7 to 99.7	4.3	87.3	99.9
	Overall	1,713	98.7 (78/79)	93.1 to 100	99.4 (1,624/1,634)	98.9 to 99.7	4.6	88.6	99.9
	Difference		1.000[h,i]	−1.9 to 6	1.00[h,i]	−0.68 to 0.88			
ES	Symp	582	100 (30/30)	90.5 to 100	99.8 (551/552)	99.0 to 100	5.1	96.8	100
	Asymp	1,128	95.8 (46/48)	85.7 to 99.5	99.4 (1,074/1,080)	98.8 to 99.8	4.3	88.5	99.8
	Overall	1,710	97.4 (76/78)	91.0 to 99.7	99.6 (1,625/1,632)	99.1 to 99.8	4.6	91.6	99.9
	Difference		0.520[h,i]	−1.5 to 9.8	0.434[h,i]	−0.19 to 0.90			
UR-F	Symp	582	100 (31/31)	90.8 to 100	99.8 (550/551)	99.0 to 100	5.3	96.7	100
	Asymp	1,136	96.1 (49/51)	86.5 to 99.5	99.8 (1,083/1,085)	99.3 to 100	4.5	96.1	99.8
	Overall	1,718	97.6 (80/82)	91.5 to 99.7	99.8 (1,633/1,636)	99.5 to 100	4.8	96.4	99.9
	Difference		0.524[h,i]	−1.4 to 9.2	1.000[h,i]	−0.43 to 0.44			
UR-M	Symp	254	96.1 (50/52)	86.8 to 99.5	100 (202/202)	98.5 to 100	20.5	100	99.0
	Asymp	1,132	100 (29/29)	90.2 to 100	99.9 (1,102/1,103)	99.5 to 100	2.6	96.7	100
	Overall	1,386	97.5 (79/81)	91.4 to 99.7	99.9 (1,304/1,305)	99.6 to 100	5.8	98.7	99.8
	Difference		0.535[h,i]	−9.1 to 1.38	1.000[h,i]	−0.087 to 0.27			

[a] VS, vaginal swab; ES, endocervical swab; UR-F, urine sample, female; UR-M, urine sample, male.
[b] Symp, symptomatic; Asymp, asymptomatic.
[c] Sens, sensitivity.
[d] Spec, specificity.
[e] Prev, prevalence.
[f] PPV, positive predictive value.
[g] NPV, negative predictive value.
[h] A 2-sample unpaired *P* test consistently showed a Fisher's exact *P* value much greater than 0.05 for all cases, as well as a 95% CI on the differences, which includes zero.
[i] *P* value.

TABLE 3 Performance of Xpert CT/NG versus patient infection status by symptomatic status for *Neisseria gonorrhoeae*

Sample type[a]	Symptom status[b]	Total (*n*)	Sens[c] (% [no. positive/no. total])	95% CI	Spec[d] (% [no. positive/no. total])	95% CI	Prev[e] (%)	PPV[f] (%)	NPV[g] (%)
VS	Symp	581	100 (10/10)	74.1 to 100	99.8 (570/571)	99.0 to 100	1.7	90.9	100
	Asymp	1,132	100 (12/12)	77.9 to 100	99.9 (1,119/1,120)	99.5 to 100	1.1	92.3	100
	Overall	1,713	100 (22/22)	87.3 to 100	99.9 (1,689/1,691)	99.6 to 100	1.3	91.7	100
	Difference		1.000[h,i]	−0.001 to 0.001	1.000[h,i]	−0.47 to 0.23			
ES	Symp	582	100 (10/10)	74.1 to 100	100 (572/572)	99.5 to 100	1.7	100	100
	Asymp	1,128	100 (12/12)	77.9 to 100	100 (1,116/1,116)	99.7 to 100	1.1	100	100
	Overall	1,710	100 (22/22)	87.3 to 100	100 (1,688/1,688)	99.8 to 100	1.3	100	100
	Difference		1.000[h,i]	−0.001 to 0.001	1.000[h,i]	−0.0001 to 0.0001			
UR-F	Symp	582	100 (11/11)	76.1 to 100	100 (571/571)	99.5 to 100	1.9	100	100
	Asymp	1,136	91.7 (11/12)	61.5 to 99.8	99.9 (1,123/1,124)	99.5 to 100	1.1	91.7	99.9
	Overall	1,718	95.6 (22/23)	78.1 to 99.9	99.9 (1,694/1,695)	99.7 to 100	1.3	95.6	99.9
	Difference		1.000[h,i]	−7.3 to 24	1.000[h,i]	−0.1 to 0.26			
UR-M	Symp	254	97.8 (44/45)	88.2 to 99.9	100 (209/209)	98.6 to 100	17.7	100	99.5
	Asymp	1,132	100 (5/5)	54.9 to 100	99.9 (1,126/1,127)	99.5 to 100	0.4	83.3	100
	Overall	1,386	98.0 (49/50)	89.4 to 99.9	99.9 (1,335/1,336)	99.6 to 100	3.6	98.0	99.9
	Difference		1.000[h,i]	−6.5 to 2.1	1.000[h,i]	−0.1 to 0.2			

[a] VS, vaginal swab; ES, endocervical swab; UR-F, urine sample, female; UR-M, urine sample male.
[b] Symp, symptomatic; Asymp, asymptomatic.
[c] Sens, sensitivity.
[d] Spec, specificity.
[e] Prev, prevalence.
[f] PPV, positive predictive value.
[g] NPV, negative predictive value.
[h] A 2-sample unpaired *P* test consistently showed a Fisher's exact *P* value much greater than 0.05 for all cases, as well as a 95% CI on the differences, which includes zero.
[i] *P* value.

females, the estimates were 95.6% and 99.9%, respectively. For urine specimens from males, the sensitivity and specificity estimates were 98.0% and 99.9%, respectively.

(iii) Assay-to-assay comparisons. Tables 4 and 5 show test comparisons for each assay using the PIS determined by each of the two assays not being evaluated. Thus, the BDPT was compared to the PIS estimated based on Xpert and AC2 results, while AC2 was compared to the PIS created from Xpert and BDPT results. Detailed information regarding the female chlamydia and gonorrhea results obtained by sample type for each assay is shown in the supplemental material. No statistical differences in performance estimates were identified among the three assays (all *P* values were >0.5). Therefore, the Xpert performance characteristics were equivalent to those of the two comparator assays using data from this trial for FDA clearance, which was received in December 2012. Finally, to help visualize the relative performance of the three assays, we utilized Venn diagrams (Fig. 2) for comparisons of the positive results for men and women.

DISCUSSION

In this multicenter trial, we evaluated a new easy-to-use cartridge-based NAAT assay platform, the Cepheid GeneXpert CT/NG assay, for the real-time simultaneous detection of chlamydia and gonorrhea. Based on two comparator assays for 1,722 female and 1,387 male patients, clinical sensitivity and specificity were excellent for all specimen types, including self-administered vaginal swabs. Results for *C. trachomatis* demonstrated sensitivities for endocervical, vaginal, and urine samples from females of 97.4%, 98.7%, and 97.6%, respectively, and a sensitivity in urine samples from males of 97.5%, with near-perfect specificities. Results for *N. gonorrhoeae* demonstrated sensitivities for endocervical, vaginal, and urine samples from females of 100.0%, 100.0%, and 95.6%, respectively, and a sensitivity in urine samples from males of 98.0%, again with near-perfect specificities. The assay performed well for both asymptomatic and symptomatic patients and thus is useful for both diagnosis and screening.

Our data once again demonstrate that the presence or absence of symptoms has little impact on test performance (12, 13), as well as that both *C. trachomatis* and *N. gonorrhoeae* infections are regularly asymptomatic, particularly in women. For *C. trachomatis* and *N. gonorrhoeae*, there was no statistical difference in test sensitivity for men or women for any sample type when participants were stratified based on symptoms. Although *C. trachomatis* sensitivity for urine, as the recommended sample of choice for testing men, was slightly lower for symptomatic men (96.1%) than for asymptomatic men (100%), the difference was not statistically significant (2, 14). For females, although *N. gonorrhoeae* sensitivity for female urine was slightly lower for asymptomatic women (91.7%) than for symptomatic women (100%), the difference was not statistically significant.

It is of note that there was no statistical difference between asymptomatic and symptomatic women overall for *C. trachomatis* or *N. gonorrhoeae*; however, the study might not have been powered to detect such a difference for only *N. gonorrhoeae*, since there was a small number of positive *N. gonorrhoeae* samples tested. There was an approximately 5%-lower sensitivity for urine samples for *N. gonorrhoeae* and an approximate 20% range for the 95% confidence intervals. Other studies have also noted lower sensitivities for urine samples (7, 11, 13, 15, 16).

PIS, defined by having positive test results from two different commercially available platforms, the Gen-Probe APTIMA Combo 2

TABLE 4 Rolling patient infection standard status of nucleic acid amplification assays for specimens from females

Assay performance characteristic[a]	Assay comparison[b]					
	Xpert vs ProbeTec ET/APTIMA Combo 2		ProbeTec ET vs Xpert/APTIMA Combo 2		APTIMA Combo 2 vs Xpert/ProbeTec ET	
	% (no. positive/no. total)	95% CI	% (no. positive/no. total)	95% CI	% (no. positive/no. total)	95% CI
Endocervical swabs for *Chlamydia trachomatis*						
Sensitivity	97.4 (76/78)	91.0–99.7	89.4 (76/85)	80.9–95.0	96.3 (77/80)	89.4–99.2
Specificity	99.6 (1,625/1,632)	99.1–99.8	99.8 (1,614/1,617)	99.5–100	99.3 (1,613/1,625)	98.7–99.6
PPV	91.6 (76/83)	83.4–96.5	96.2 (76/79)	89.3–99.2	86.5 (77/89)	77.6–92.8
NPV	99.9 (1,625/1,627)	99.6–100	99.5 (1,614/1,623)	99.0–99.8	99.8 (1,613/1,616)	99.5–100
Accuracy	99.5 (1,701/1,710)	99.0–99.8	99.3 (1,690/1,702)	98.8–99.6	99.1 (1,690/1,705)	98.6–99.5
Endocervical swabs for *Neisseria gonorrhoeae*						
Sensitivity	100 (22/22)	84.6–100	91.3 (21/23)	72.0–98.9	95.7 (22/23)	78.1–99.9
Specificity	100 (1,688/1,688)	99.8–100	99.6 (1,673/1,680)	99.1–99.8	99.9 (1,681/1,682)	99.7–100
PPV	100 (22/22)	87.3–100	75.0 (21/28)	55.1–89.3	95.7 (22/23)	78.1–99.9
NPV	100 (1,688/1,688)	99.8–100	99.9 (1,673/1,675)	99.6–100	99.9 (1,681/1,682)	99.7–100
Accuracy	100 (1,710/1,710)	99.8–100	99.5 (1,694/1,703)	99.0–99.8	99.9 (1,703/1,705)	99.6–100
Urine samples for *C. trachomatis*						
Sensitivity	97.6 (80/82)	91.5–99.7	89.4 (76/85)	80.9–95.0	97.5 (78/80)	91.3–99.7
Specificity	99.8 (1,633/1,636)	99.5–100	99.6 (1,605–1,611)	99.2–99.9	99.7 (1,620/1,625)	99.3–99.9
PPV	96.4 (80/83)	89.8–99.3	92.7 (76/82)	84.8–97.3	94.0 (78/83)	86.5–98.0
NPV	99.9 (1,633/1,635)	99.6–100	99.4 (1,605–1,614)	98.9–99.7	99.9 (1,620/1,622)	99.6–100
Accuracy	99.7 (1,713/1,718)	99.3–99.9	99.1 (1,681/1,696)	98.6–99.5	99.6 (1,698/1,705)	99.2–99.8
Urine samples for *N. gonorrhoeae*						
Sensitivity	95.6 (22/23)	78.1–99.9	81.8 (18/22)	59.7–94.8	91.3 (21/23)	72.0–98.9
Specificity	99.9 (1,694/1,695)	99.7–100	99.9 (1,671/1,673)	99.6–100	100 (1,682/1,682)	99.8–100
PPV	95.6 (22/23)	78.1–99.9	90.0 (18/20)	68.3–98.8	100 (21/21)	84.0–100
NPV	99.9 (1,694/1,695)	99.7–100	99.8 (1,671/1,675)	99.4–99.9	99.9 (1,682/1,684)	99.6–100
Accuracy	99.9 (1,716/1,718)	99.6–100	99.7 (1,689/1,695)	99.2–99.9	99.9 (1,703/1,705)	99.6–100

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] Xpert, Cepheid GeneXpert CT/NG assay; ProbeTec ET, Becton, Dickinson ProbeTec ET assay; APTIMA Combo 2, Gen-Probe APTIMA Combo 2 assay.

and the Becton, Dickinson ProbeTec, was useful for evaluating the overall performance of the Xpert real-time PCR assay. A definition of PIS based on two different comparator platforms provides conservative estimates of performance and has the possible effect of providing slightly higher sensitivity and slightly lower specificity estimates than if two sample types from any one assay type are used (5), as has been done for earlier diagnostic trials for chlamydia and gonorrhea (13, 16, 17). The rolling patient infection status analysis provided interesting

TABLE 5 Rolling patient infection standard status of nucleic acid amplification assays for urine specimens from males

Assay performance characteristic[a]	Assay comparison[b]					
	Xpert vs ProbeTec ET/APTIMA Combo 2		ProbeTec ET vs Xpert/APTIMA Combo 2		APTIMA Combo 2 vs Xpert/ProbeTec ET	
	% (no. positive/no. total)	95% CI	% (no. positive/no. total)	95% CI	% (no. positive/no. total)	95% CI
Urine samples for *Chlamydia trachomatis*						
Sensitivity	97.5 (79/81)	91.4–99.7	93.8 (75/80)	86.0–97.9	98.7 (78/79)	93.2–100
Specificity	99.9 (1,304/1,305)	99.6–100	99.6 (1,295/1,300)	99.1–99.9	99.8 (1,295/1,298)	99.3–99.9
PPV	98.7 (79/80)	93.2–100	93.8 (75/80)	86.0–97.9	96.3 (78/81)	89.6–99.2
NPV	99.9 (1,304/1,306)	99.5–100	99.6 (1,295/1,300)	99.1–99.9	99.9 (1,295/1,296)	99.6–100
Accuracy	99.8 (1,383/1,386)	99.4–100	99.3 (1,370/1,380)	98.7–99.7	99.7 (1,373/1,377)	99.3–99.9
Urine samples for *Neisseria gonorrhoeae*						
Sensitivity	98.0 (49/50)	89.4–99.9	98.0 (48/49)	89.2–100	100 (49/49)	92.8–100
Specificity	99.9 (1,335/1,336)	99.6–100	99.7 (1,327/1,331)	99.2–99.9	99.9 (1,324/1,325)	99.6–100
PPV	98.0 (49/50)	89.4–100	92.3 (48/52)	81.5–97.	98.0 (49/50)	89.4–99.9
NPV	99.9 (1,335/1,336)	99.6–100	99.9 (1,327/1,328)	99.6–100	100 (1,324/1,324)	99.7–100
Accuracy	99.9 (1,384/1,386)	99.5–100	99.6 (1,375/1,380)	99.2–99.9	99.9 (1,373/1,374)	99.6–100

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] Xpert, Cepheid GeneXpert CT/NG; ProbeTec ET, Becton, Dickinson ProbeTec ET; APTIMA Combo 2, Gen-Probe APTIMA Combo 2.

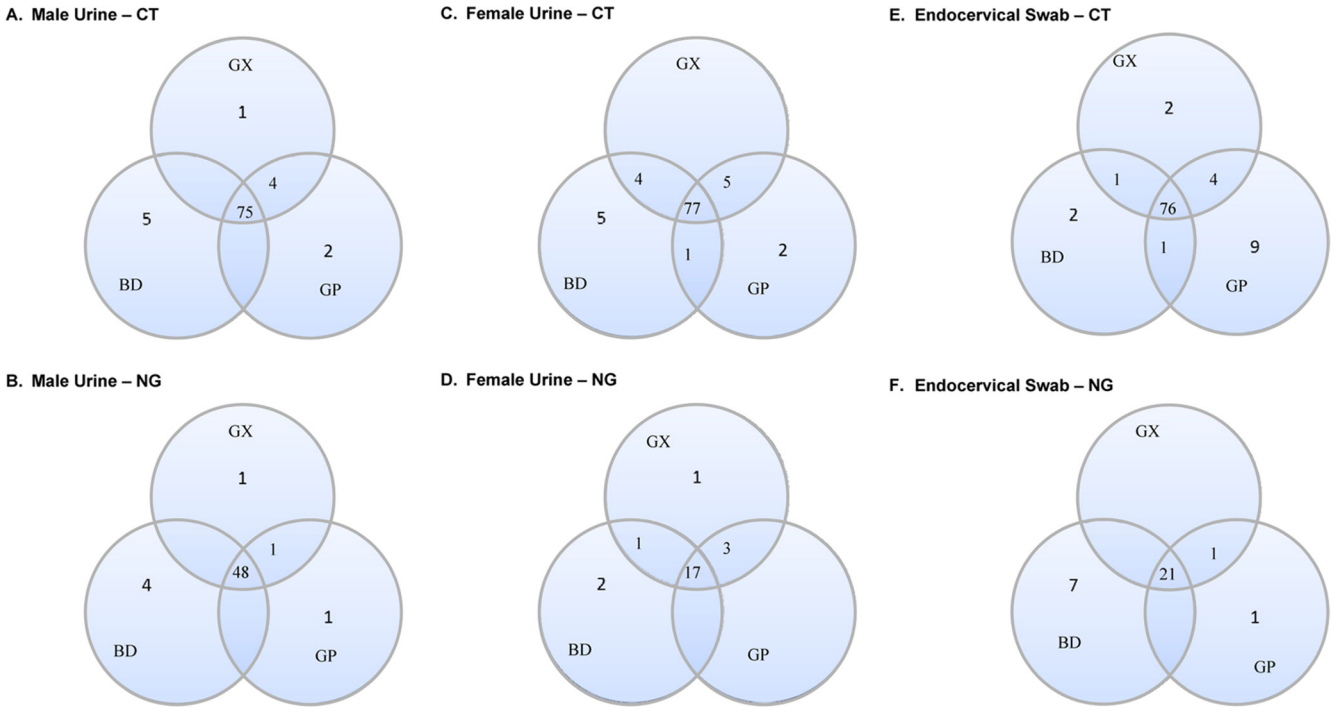

FIG 2 Venn diagrams showing concordance of positive samples for Xpert (Cepheid GeneXpert CT/NG), BD (Becton, Dickinson ProbeTec ET), and GP (Gen-Probe APTIMA Combo 2) for all sample types for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG).

results that demonstrated that the Xpert assay is as sensitive and specific as the two comparator FDA-cleared commercial NAAT assays.

Data analysis by the Venn diagrams provided an interesting perspective, which indicated that while agreement between the three tests was high, no assay type is perfect, and that only a small number of tests were positive for any one assay type. Although Xpert results were generated for 97.1% of samples on the first test, occasionally this result was invalid due to an indeterminate result, requiring a second test. The reason is unknown; perhaps it might have been due to microfluidic errors, but most retests were valid, giving a 99.6% assay success rate.

Of interest was the finding that there were three (3.7%) chlamydia-infected female patients with positive urine specimens, but not endocervical swabs, in all three assays. These participants were considered to be infected based on urine samples but were considered to be uninfected based on the vaginal swab sample analysis. They were all chlamydia negative according to the vaginal Cepheid Xpert vaginal swab. Since there were no vaginal swab samples collected for the Becton, Dickinson or Gen-Probe tests, these samples were considered to be uninfected for the vaginal swab analysis, but one might hypothesize that these patients were truly infected with chlamydia. This apparent "urethral dysuria syndrome" due to chlamydia has been described in several reports where only urine specimens and not cervical specimens are positive (18, 19, 20, 21, 22, 23).

A particularly distinctive attribute of the Xpert platform is its easy-to-use cartridge-based format and automated sample preparation and extraction process. This makes the assay easy to use and well suited for use at sites where clinical care is provided, providing the potential to reduce the time needed to obtain test results to guide therapeutic decision-making. The hands-on time is approximately 5 min and the time for results is quick (<2 h) compared to other NAAT platforms. The ability to provide test results and treat patients before they leave the clinic or doctor's office has substantial potential to reduce complications and it might be cost-effective depending on the cost of the assay, the time patients are willing to wait, and the number of patients who might not return to a clinic for their result (3, 4, 24).

In summary, the Cepheid GeneXpert CT/NG assay performance was highly accurate and reproducible and can be recommended for detecting both chlamydia and gonorrhea in cervical, vaginal, and urine specimens from women and in urine specimens from men. Nucleic acid amplification tests are the most sensitive assays available to date for detecting chlamydia and gonorrhea in clinical specimens, and the Xpert assay adds to the group of commercially available assays that are available to laboratories as choices for superior diagnostic performance. Also, the rapidity of the assay makes it valuable for clinic-based testing, as the results can be used to immediately treat infected patients in many cases.

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